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Proposal: The Antibody-catalyzed regioselective protection of (β-1-6) D-Galactose-D-Mannose Disaccharide as a Showcase of the use of Antibodies as Catalysts in Organic Solvents in Oligosaccharide Chemistry

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I. Introduction

With the ever-increasing importance of carbohydrates in general, and oligosaccharides, in particular, in biopharmaceuptical and biotechnological solutions, a constant demand for new synthetic tools that deal with oligosaccharides is created, particularly tools with various selective properties. Due to their inherent exquisite catalytical properties, enzymes have been explored, and have been successfully utilized in many cases(1). However, enzyme catalysis faces the constant problem of a lack of diversity in naturally occuring specimens. This paper will propose a solution to the topic using the natural diversity of antibodies and the power of the vaccination selection mechanism, while concentrating on a simple reaction to showcase both the possibilities and the challenges that such an approach faces.

II.The Abzyme Approach

The catalytic antibody approach, or the 'abzyme' approach, while still being a mostly undeveloped field in terms of commercial applications, shows great promise in the scope of uses, due to the nature of somatic recombination, the process that gives rise to the great variety of antibodies, and has the ability to biogically synthesize antibodies in a manner that we can control through the vaccination process. While many various applications of these have been explored, and the general procedure in their creation and harvesting has been already widely described in literature(2), this paper will include a short schematic explanation of the function of the antibody, and the logic and technique leading to it's application as a catalyst, as to put the proposal into the correct context.

The general logic of the Abzyme approach is that if antibodies have a biological function to capture and attach themselves to certain molecular structures, that attachment is favoured thermodynamically, and thus, if vaccination occurs with a molecule that has similar steric and electronic properties to the transition state of the rate-determining state of a reaction, the result will be an enzyme that will be able to lower the energy of the transition state of said reaction, and thus will be an effective catalyst.

III.The Galactose Mannose Disaccharide Acetalation reaction

The acetalation reaction is a staple protection reaction in both carbohydrate chemistry, and in organic chemistry in general. It's the reaction between a cis 1,2-diol group, and a carbonyl to form a acetal ring. The parallel 1,2-trans-diol reaction doesn't occur due to steric reasons. the reaction is very useful in carbohydrate chemistry in the selective protection of many monosaccharides, in order to selectively prepare them for other chemical reactions. However, while dealing with oligosaccharides, this isn't always possible, since the reaction can unselctively occur in several locations. For example, in our case both the Galactose and the Mannose units present cis-dihydroxy regions in which such a reaction can occur (fig. 1)

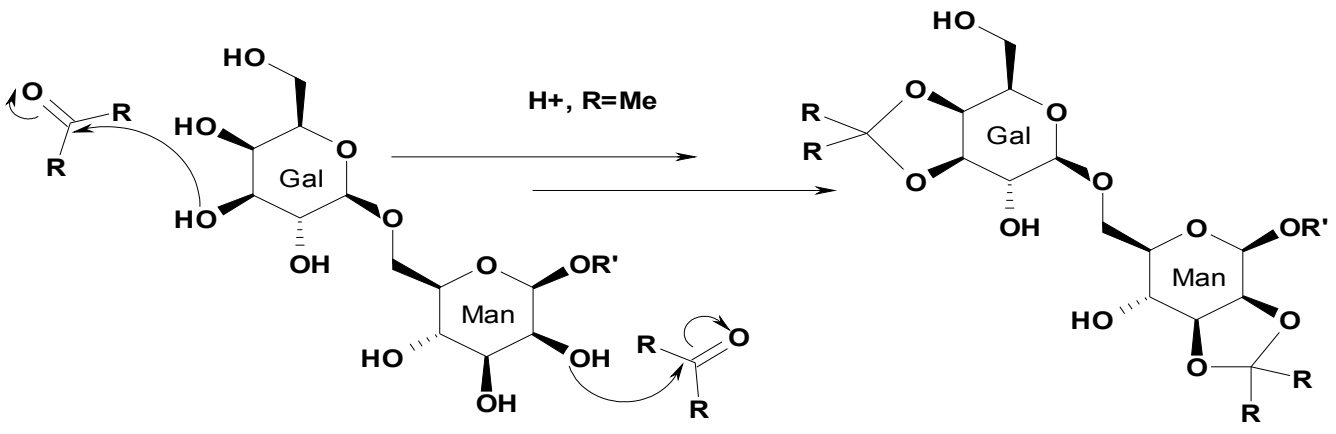

fig. 1: the reaction proceeds at both the of the units of the disaccharide.

IV.General Reaction Mechanism

The regular acetalation reaction mechanism, while simple, is crucial to understand the problems that antibody catalysis faces in this case. The mechanism can be either Brønsted acid catalyzed or Lewis Acid catalyzed (fig. 2). The mechanisms are in essense very similar, in the fact that in both, the ketone is polarized, and is more accomodating for a nucleophilic attack. However, a the Brønsted acid path includes a protonation of the carbonyl oxygen, when protonation of the attacking hydroxy group is much more suitable for protonation.Here we can easily one of the major problems we'll be facing – an equilibrium reaction with water means that the reaction cannot proceed in a simple water medium.

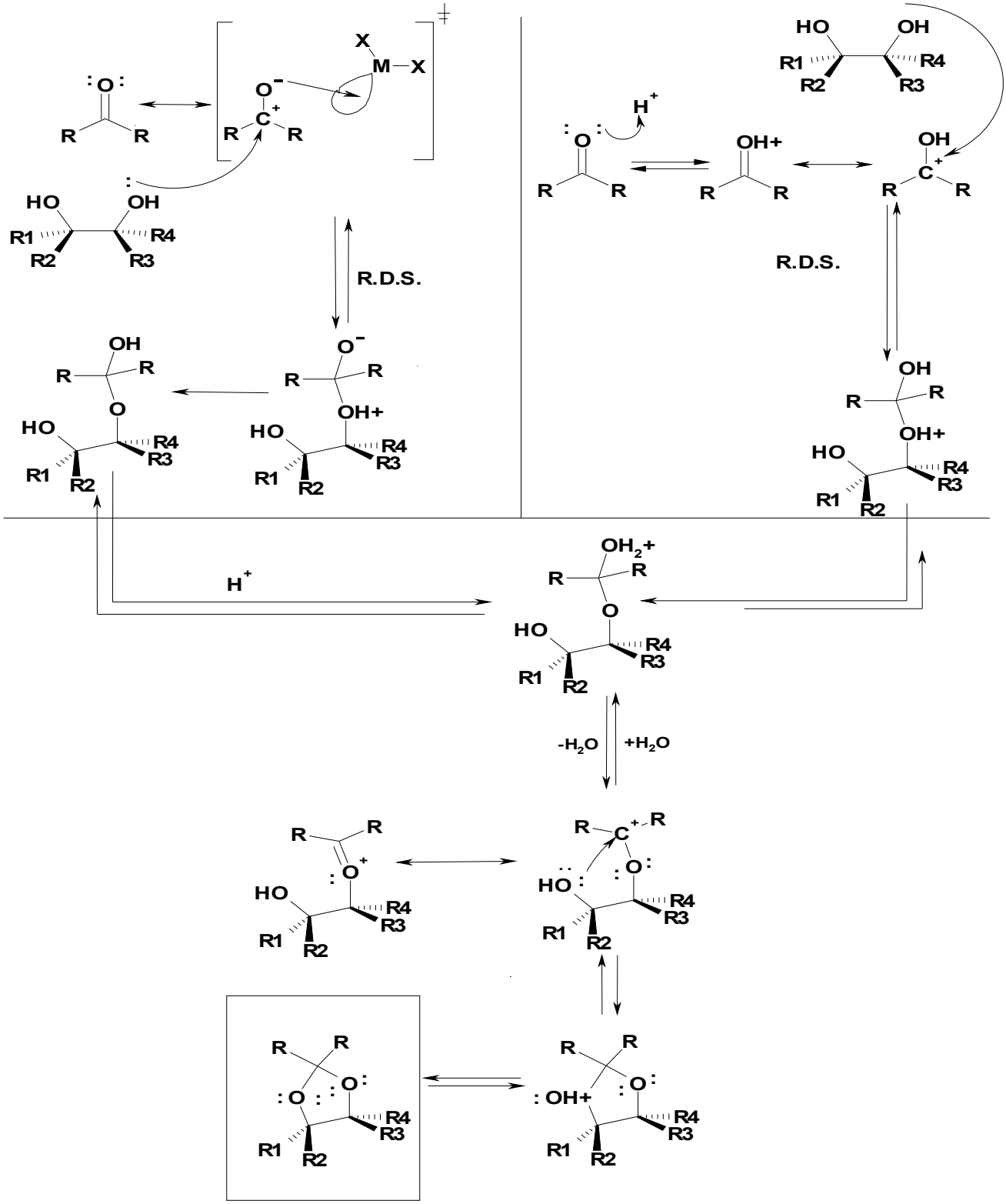

fig.2 The Brønsted and Lewis acid catalyzed mechanisms, where R_1,R_2,R_3,R_4 are the carbohydrate and hydrogens in our case.

V.Establishing the Rate-Determining Step

As we described in part II, antibody catalysis requires vaccination with a molcule that is similar in it's stereometry and electronic properties to the transition state of the rate determining step in the reaction that we wish to catalyze. Before designing the molecule determining the rate defining step is necessary. As fig.2 shows, the initial hydroxyl attack on the carbonyl was decided on as the rate determining step:
The considerations for this are the following: There are three sorts of steps here, the initial attack of the hydroxyl, proton migration and protonation/deprotonation, and the nucleophilic closing of the ring, with water as the leaving group. From these, the initial attack, a bi-molecular reaction, will be the rate determining step, as opposed to ring closing, the intramolecular reaction, due to considerations of entropy.

VI.The Hydrolysis equilibrium,denaturation problems, and their solutions

The more we get into the reaction, the more clearly we can see the problems that present themselves in this reaction: The first one is the problem that this reaction should be carried in a dry medium so that we wouldn't cause the counter reaction, which is the hydrolysis of the acetal ring. The second one, or rather the second side of the same problem, is the danger of the denaturation of the antibody in alternative non-aqueous solutions, as with any protein. Two approaches may be used as possible remidies to the issues at hand.

VII.The two-phase approach

In this approach, we begin with both the unprotected disaccharide and the antibody reside in the aqueous phase, with the ketone residing mostly in the organic phase. However, the ketone will be somewhat water soluble, and will migrate between the two phases. With the reaction occuring, the solubility of the product will fall dramatically, so that it will migrate to the aqueous phase, and will then not be subject to hydrolysis.

It is possible to choose the appropriate reaction parameters in such a way that the reaction will be able to take place in a two phase enviroment. For this, the organic phase must be as least water soluble as possible so that water wouldn't enter it, and very importanly, it wouldn't enter the aquatic phase as well, so that it wouldn't inhibit the catalytic properties of the protein through partial denaturation.Also, the ketone alkyl groups must be both large, and branch out , so that it would lower the product solubility the most dramatically, and would protect both the acetal ring from hydrolysis and the unprotected hydroxyl ends from hydrogen bonds as much as possible. On the other hand, the ketone still must be very slightly water soluble . The most suitable candidates seem to be diethyl ketone, 2,4-dimethyl-pentan-3-one and 2,6-dimethyl-hepta-4-none. However, while they will indeed mostly be bulky enough to move the product into the organic phase, it's possible that their water solubility will be too low to have a high enough reaction rate, even considering the dramatically increased reactivity that is achieved through the addition of the catalyst. An additional problem is whether the water solubility of the disaccharide is now low enough for it to go to the organic phase. Here, this can be solved by easily, selectively pre-placing a bulky alkyl group on the C6 of Gal (fig.3)
(Bulky alkyl halides are highly selective towards the sixth carbon of carbohydrates, and form stable ethers). Thus, an addition of an acetal ring with two alkyl chains instead of two hydroxyl groups, will mostly likely tip the dissacharide into the organic phase. Generally, the rule of thumb would be the larger the oligosaccharide, the larger the corresponding alkyl group will have to be.

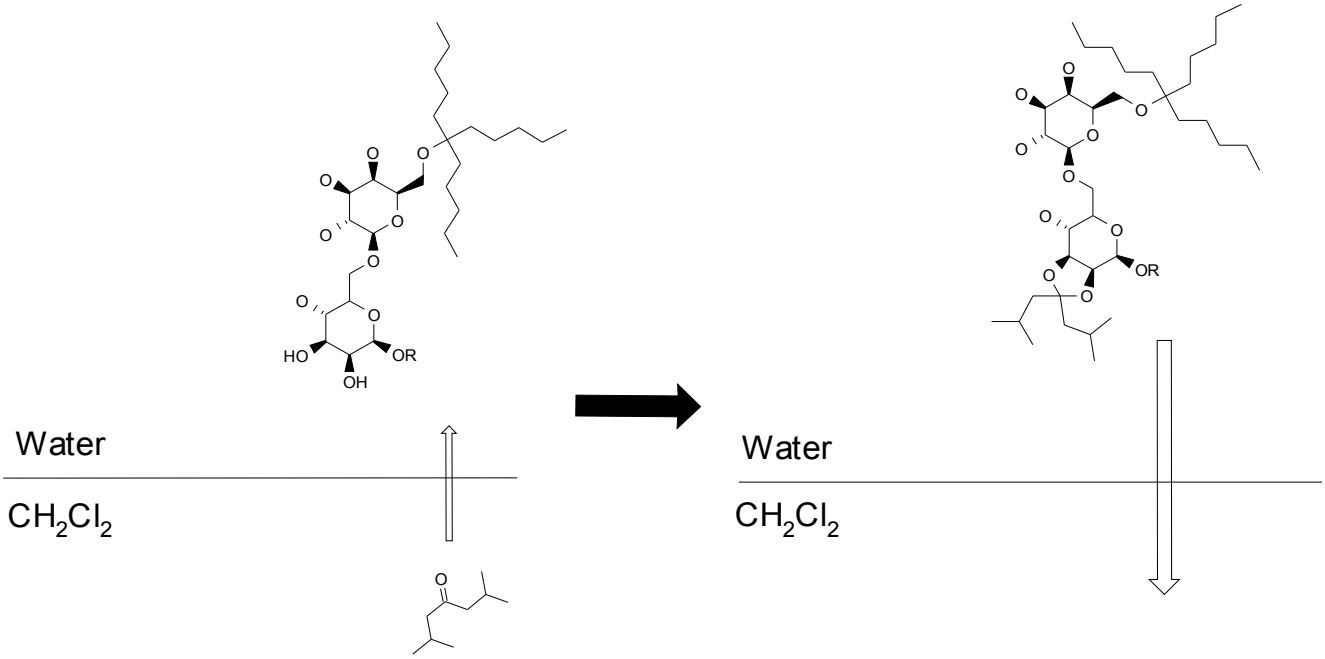

Fig. 3 schematic representation of the two-phase approach with the Gal-Man disaccharide, 2,4-dimethyl-pentan-3-one as the protecting group, and 6-pentyl-6-undecanyl as the hydrophobic anchor.

VIII.Exploring the possibility of an alternative approach in larger oligosaccharides through the application of the catalytical antibody in organic solvents

While the two phase approach relies on some rather basic premises, such as changes in solubility, with the antibody catalyst remaining firmly in the aqueous phase at all times, this approach has two limitations on it, like the maximum size of the oligosaccharide, since an oligosaccharide too large wouldn't be influenced enough by a small protecting ketone, and a large protecting ketone wouldn't be water soluble enough to react. Another limitation is the relative delicacy of the conditions, and an almost total phase separation, since a large amount of intermixing between the two phases may cause both hydrolysis, and protein denaturation. Thus, the two phase method often faces challenges that can keep the scope of it's application rather limited.

An alternative approach can be explored by using the catalysts in complete organic solutions. At first, this goes against conventional logic, that dictates that proteins are denatured in organic solvents. This, however, was shown not to be the case for pure organic solvents, but in organic-aqueous mixtures, due to the lack of protein flexibility provided by water molecules[(3)]. It has been shown that through lyophilization, and subsequent intoduction to an organic non-polar solvent, it is possible to retain the protein in a rigid native form. This technique has been explored in enzymes, and has shown signs of keeping, and even, at times, improving the catalytical properties of enzymes[(4)]. It is fair to assume that antibodies will display a similar array of responses to this set of procedures. Additionally, similarly to procedures explored in enzymes [(5)], an introduction of the transition state analog during the process of lyophilization can be used to improve and ensure the catalytical properties of the antibody after the lyophilization, a process known as Biomolecular Imprinting. Thus it there is also a possibility of designing proper conditions for this catalysis in an organic solvent: the solvent must be non-polar, so that it won't be a molecular lubricant for denaturation, very small amounts of polar solvent might be needed in to give a small amount of structural flexibility to the protein – a setting in which acetone may not only be non-harmful, but actually welcomed as the protecting ketone reactant.

IX.Designing the right Haptene for the job

With the specific issues of the application of catalytical antibodies in an organic solvent adressed, we can return to the issue of the design of the Transition State Analog, TSA, or Haptene, as it's also known. As explained in part V, the rate determining step is the attack of the hydroxy lone pair on the ketone carbonyl, with the ketone molecule previously polyrized by stabilizing a negative charge in the ketone oxygen. Another consideration is the regioselectivity aspect. Thus, in the haptene, only one of the saccharides, the one we desire to be protected should be altered. In this paper, the proposition is to protect the Mannose. Thus the proposed hapten will be this:

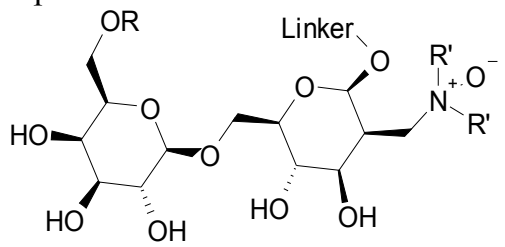

fig.4 The proposed Haptene for the protection reaction. R=H or a series or an aliphatic anchor if the two phase procedure is followed.R' = Me or a larger aliphatic group, depending on the choice of the protecting ketone.

X.Synthesis of the Haptene

The synthesis of the haptene is a multi-stage procedure which includes mainly standard carbohydrate chemistry. Here is a proposed synthesis from basic sugars.

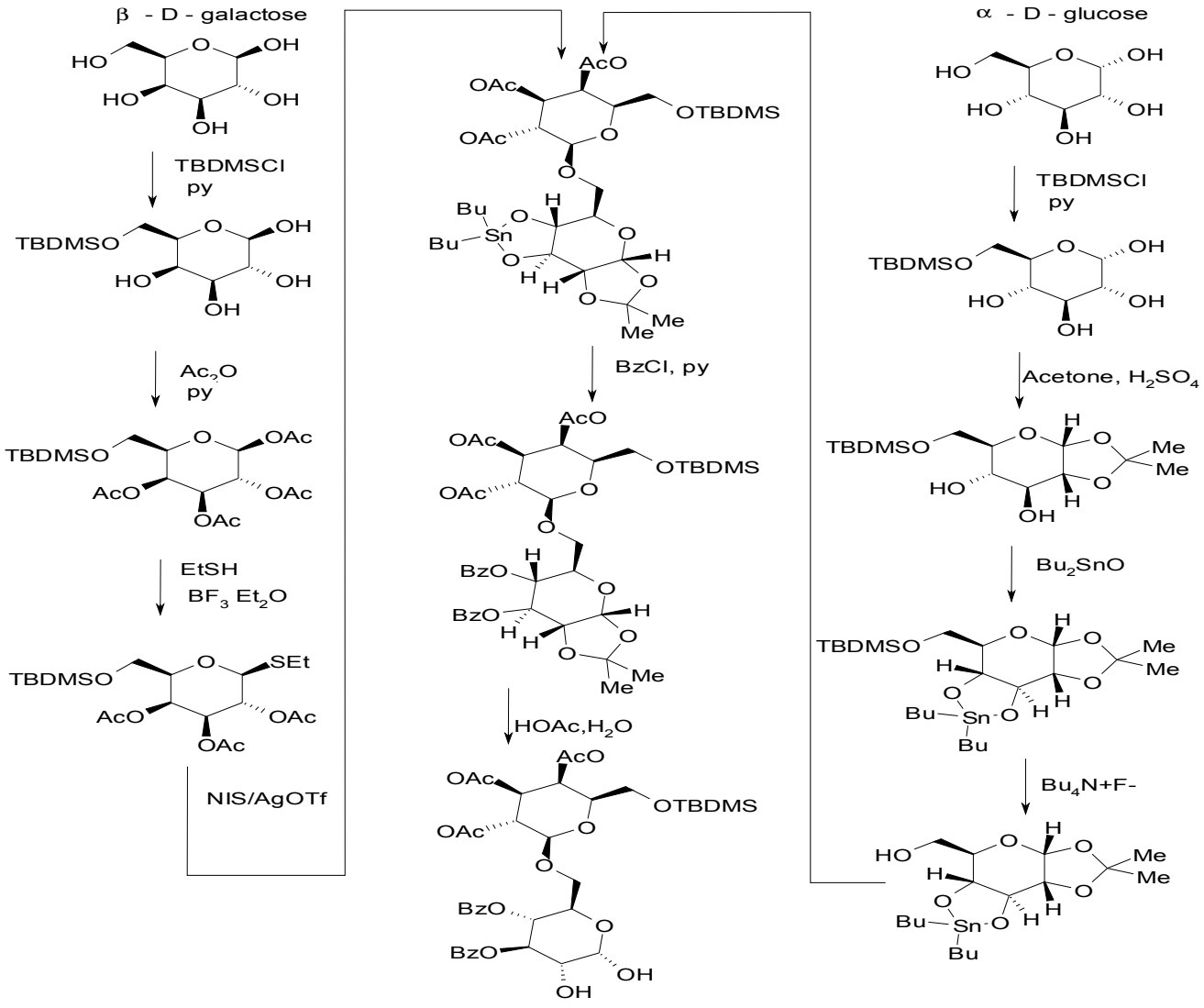

fig.5A The beginning stages of the synthesis of the haptene

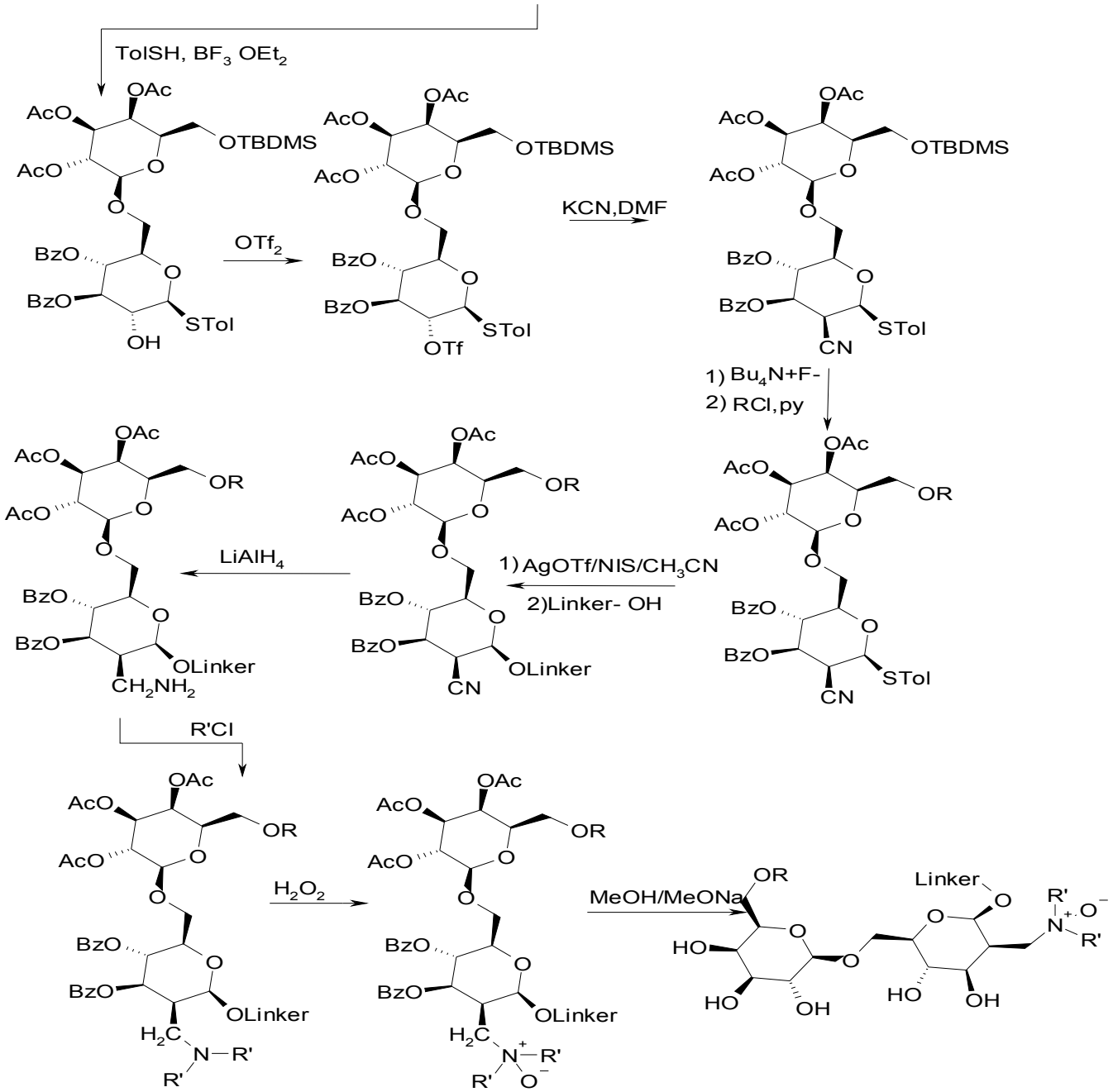

fig. 5B The final stages of the haptene synthesis.

XI.Conclusion

While the possibility of use of catalytic antibodies in carbohydrate and oligosaccharide chemistry faces many difficulties, this paper showcases that such a nieche of activity for antibodies may become viable as the whole area of antibody catalysis grows. Only time will show the possiblity of this, with the possible facilitation by newer protein technologies, and dependant on the demand for such catalysis by industry and science.

References:

(1) Ichikawa Y, Look GC, Wong CH, *Anal Biochem*. 1992 May 1;202(2):215-38.
(2)E. Keinan ,*Catalytic Antibodies* , Wiley, ISBN: 3-527-30688-9
(3)Griebenow K, Klibanov A.M., *J.Am. Chem. Soc.* 47, 11695-11700, 1996
(4)M.Klibanov, *Nature*, 409, 241-246
(5)Christopher J. Slade, Evgeny N. Vulfson, *Biotechnology and Bioengineering*, Vol.57,Iss.2, 211-215